Trends in Endocrinology & Metabolism

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Review

Neuronal Control of Brown Fat Activity

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Brown adipose tissue (BAT) activation reduces body fat and metabolic disorders by the enhanced combustion of lipids and glucose into heat. The thermogenic activity of brown adipocytes is primarily driven by the sympathetic nervous system (SNS) and controlled by the brain. In this review, we present recent advances in understanding how cues, such as temperature, light, and proteins, modulate the activity of brown fat by acting on the various hypothalamic nuclei. Given that activated BAT has a high capacity to take up and burn fatty acids (FAs) and glucose, pharmacological stimulation of brown fat in humans by either targeting the hypothalamus or mimicking outflow of the sympathetic nervous system might help improve glucose metabolism and insulin sensitivity, and also lower body fat.

Trends

Brown fat thermogenesis is primarily driven by the sympathetic nervous system.

Multiple hypothalamic nuclei are crucially involved in regulating brown fat activity. This underscores the physiological importance of brown fat for the body.

Cold exposure increases rodent and human brown fat activity, an effect that is efficiently mimicked by β3 adrenergic receptor agonists.

Activation of brown fat protects from obesity and related disorders.

Hypothalamic Regulation of Energy Expenditure

One of the first studies confirming a crucial role of the nervous system in energy homeostasis dates back to 1942, when Hetherington and Ranson [1] described how hypothalamic lesioning of rats frequently caused obesity, thereby providing evidence for the control of food intake and energy expenditure by a specific brain region. However, the mechanism by which the hypothalamus regulated energy expenditure remained elusive.

Today, the central and peripheral nervous systems (CNS and PNS) are increasingly recognized as the regulators of energy expenditure. Many hypothalamic circuits are involved in the regulation of energy expenditure, including the melanocortin system, among others [2]. The (re-)discovery of BAT in small mammals and humans [3–5] shifted the research focus to the role of the nervous system in beige and brown fat-mediated energy expenditure as possible treatment targets in obesity and related disorders, including type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD). Here, we review recent advances in elucidating the neuronal control of the physiology of beige and brown fat, and discuss treatment possibilities that either target the CNS or mimic outflow of the PNS to induce activation of brown fat and/or browning of white fat.

Physiology of Brown Fat

While the main function of white adipose tissue (WAT) is to store energy in the form of triglycerides (TG), BAT combusts TG into heat, a process referred to as 'adaptive thermogenesis'. Brown fat depots are strategically localized in the scapular area near the large arteries, where heat production appears to be essential for the survival of small mammals in cold environments and for arousal of hibernators [6]. In addition to its crucial role in nonshivering thermogenesis, BAT is probably also required for maintaining energy balance and is activated upon overeating, a process called 'diet-induced thermogenesis'. However, the existence of such a process is a matter for debate [7].

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BAT is characterized by multilocular intracellular lipid droplets, numerous mitochondria, and regulated expression and activity of uncoupling protein 1 (UCP1). Clusters of UCP1-expressing adipocytes with thermogenic capacity also develop in WAT in response to various stimuli, including cold exposure and β-adrenergic agonists. These adipocytes have been named beige, brite, or recruitable brown adipocytes and have characteristics that distinguish them from classical brown adipocytes. Controversy exists around the nature of these beige adipocytes and whether, in adult humans, BAT represents mainly brown or beige adipocytes [8].

The thermogenic activity of brown fat is primarily driven by the SNS, reflected by a high density of nerve endings in the tissue. Norepinephrine-induced adrenergic receptor signaling enhances expression of proteins involved in thermogenesis and stimulates intracellular lipolysis [6]. Liberated FAs are directed to the mitochondria to be combusted, or may allosterically activate UCP1 [9]. This results in the uncoupling of complexes I–IV of the respiratory chain and ATP synthesis by proton leakage from the mitochondrial inner-membrane space into the mitochondrial matrix, thereby generating heat instead of ATP (Figure 1). Long-term sympathetic outflow towards the fat depots additionally leads to an increased mass of BAT depots and so-called 'browning' of WAT [10]. Since activated BAT has a high capacity to take up and burn TG-derived FAs and glucose, BAT is considered a promising target to combat obesity and associated diseases, including T2DM and CVD.

Autonomic Innervation of Brown Fat

Separate populations of pre-autonomic nerve fibers from hypothalamic nuclei relay to either parasympathetic or sympathetic nuclei in the brain stem and spinal cord, respectively. While the primacy of BAT activation by the SNS is clear and all rodent BAT depots are innervated by sympathetic nerve fibers, the role of the parasympathetic nervous system in BAT is less well known. Only the minor mediastinal and pericardial BAT depots appear to receive parasympathetic innervation, as indicated by immunoreactivity for vesicular acetylcholine transporter [11,12]. However, the function of the parasympathetic nervous system in brown fat biology remains unknown.

BAT thermogenesis is activated by the SNS with the release of norepinephrine and subsequent stimulation of β-adrenergic receptors (Figure 1); mice deficient for all three subtypes of the β-adrenergic receptor are cold intolerant and obese [13]. Within BAT, the β3-adrenergic receptor is most abundant and β2-adrenergic receptors are probably restricted to the blood vessels to regulate vasodilation [14,15]. Efforts identifying the exact contribution of each type of adrenergic receptor to brown fat function are inconclusive. Mice deficient for the β1-adrenergic receptor showed a lower basal metabolic rate and defective cold tolerance [16]. Correspondingly, blockade of primarily β1- and β2-adrenergic receptors by administration of propranolol reduced BAT activity, as determined by the uptake of [^{18}F]fluorodeoxyglucose ([^{18}F]FDG) on PET imaging in mice [17] and patients with cancer [18]. By contrast, β3-adrenergic receptor-deficient mice do not exhibit defects in basal metabolic rate or cold tolerance [19] and administration of a β3-adrenergic receptor agonist increased energy expenditure, mainly through browning of WAT, rather than activation of BAT [20]. However, β3-adrenergic receptor-deficient mice do show upregulation of β1-adrenergic receptor expression in BAT, indicating that the animals compensate for insufficient thermogenesis [19]. More recent studies showed that pharmacological stimulation of specifically β3-adrenergic signaling enhanced thermogenesis in brown adipocytes [21], rodent BAT [22], and human BAT [23]. Although the β1-adrenergic receptor is probably more important in BAT physiology, most (clinical) research is focused on the development of specific β3-adrenergic receptor agonists because β1- and β2-adrenergic receptor activation coincides with cardiovascular complications.

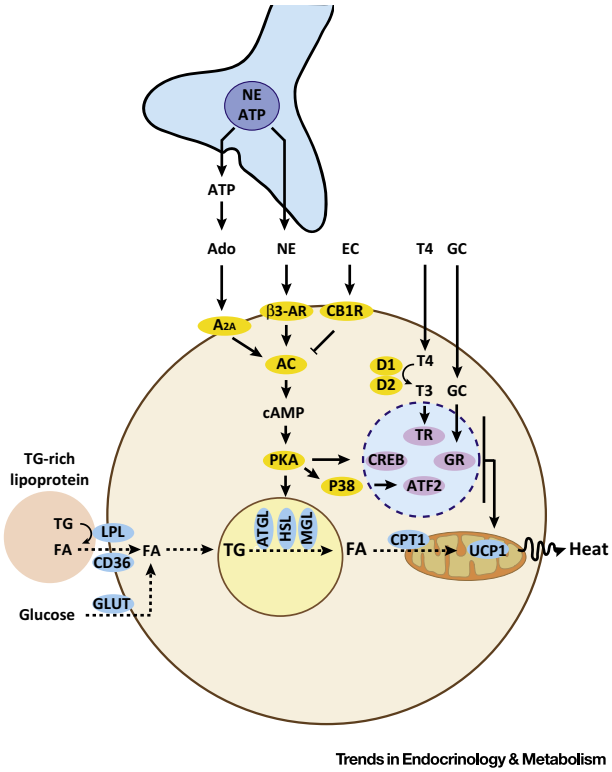

Figure 1. Activation of the Brown Adipocyte via the Sympathetic Nervous System, Hypothalamic–Pituitary–Thyroid (HPT) Axis, and Hypothalamic–Pituitary–Adrenal (HPA) Axis. Upon enhanced sympathetic activity, both norepinephrine (NE) and ATP are released from nerve endings near brown adipocytes. ATP is converted into adenosine (Ado), which activates the A_{2A} receptor. Both the β3-adrernergic receptor (β3-AR) and A_{2A} receptor stimulate cyclic AMP (cAMP) production by adenylyl cyclase (AC). By contrast, endocannabinoids (EC) may reduce this signaling through cannabinoid type 1 receptor (CB1R)-mediated inhibition of AC. cAMP activates protein kinase A (PKA), which drives lipolysis through phosphorylation of hormone-sensitive lipase (HSL) and induces transcription of genes involved in mitochondrial biosynthesis and thermogenesis via phosphorylation of cAMP response element-binding protein (CREB) and p38-mediated phosphorylation of activating transcription factor 2 (ATF2). In addition, gene expression is regulated by thyroid hormone (T4/T3) and glucocorticoid (GC) signaling. Fatty acids (FA) that are released from lipid droplets are directed towards the mitochondria to be combusted or may allosterically activate uncoupling protein 1 (UCP1). Intracellular lipid stores need to be replenished by uptake of glucose and triglyceride (TG)-derived FA from the circulation. Abbreviations: ATGL, adipose triglyceride lipase; CD36, cluster of differentiation 36; CPT1, carnitine palmitoyltransferase I; D1, type I iodothyronine deiodinase; D2, type II iodothyronine deiodinase; Glut, glucose transporters; GR, glucocorticoid receptor; LPL, lipoprotein lipase; MGL, monoacylglycerol lipase; TR, thyroid hormone receptor.

The stimulatory effect of catecholamines on BAT thermogenesis may be modulated by endocannabinoids, a group of neuromodulatory lipids with both central and peripheral functions. On the one hand, knockout of the cannabinoid type 1 receptor (CB1R) in single-minded 1 (Sim1) neurons, which account for most paraventricular nucleus (PVN) neurons, increased brown fat thermogenesis and protected from diet-induced obesity, indicating that endocannabinoids regulate BAT activity through the brain [24]. On the other hand, peripheral CB1R antagonism activated brown adipocytes directly, through relief of CB1R-mediated inhibition of cAMP production [25].

Recently, it became evident that, in addition to catecholamines, adenosine acts as a sympathetic co-transmitter and mediates part of the cold-induced activation of brown fat at least in mice, and potentially also in humans [26]. Mechanistically, stimulation of purinergic A_{2A}-adrenergic receptors on brown adipocytes induced intracellular cAMP production by adenylyl cyclase, thereby driving the same activating pathway as adrenergic signaling. Correspondingly, pharmacological stimulation of the A_{2A}-adrenergic receptors in mice activated BAT and induced browning of

WAT, resulting in improved glucose tolerance and protection from diet-induced obesity [26]. Although promising, similar to β-adrenergic receptors, purinergic receptors are also highly expressed on the cardiovascular system and immune cells [27], indicating a risk of adverse events.

Hypothalamic Innervation of Brown Fat

Retrograde viral tracing methods have been used to identify hypothalamic origins of neuronal activity in BAT. Injection of the transneuronal pseudorabies virus (PRV) in interscapular BAT (iBAT) of hamsters [28] and later also rats [29,30] revealed potential neuronal connectivity with the preoptic area (POA), paraventricular hypothalamus (PVH), dorsomedial hypothalamus (DMH), lateral hypothalamic area (LHA), and suprachiasmatic nucleus (SCN). In recent years, specific neuron types in hypothalamic areas have also been identified that regulate BAT activity. Neurons in the arcuate nucleus projecting to BAT express cocaine- and amphetamine-regulated transcript (CART), pro-opiomelanocortin (POMC), and leptin receptors, whereas BAT-innervating neurons in the LHA mainly express melanin-concentrating hormone (MCH) and orexins [30]. Functional studies showed that the thermoregulatory action of leptin is mediated through leptin receptor neurons in the POA and DMH, likely via synaptic projections to the rostral raphe pallidus neurons [31]. Furthemore, GABAergic RIP-Cre neurons in the arcuate nucleus contribute to the stimulatory effect of leptin on brown fat [32]. Involvement of other hypothalamic nuclei in thermogenesis is discussed in more detail in the next section.

The presence of nerve fibers in BAT containing sensory-associated neuropeptides also suggests sensory innervation. Correspondingly, injection of the anterograde transneuronal herpes simplex virus type 1 (HSV1) provided neuroanatomical evidence for sensory connections to the PVH, periaqueductal gray, olivary areas, parabrachial nuclei, raphe nuclei, and reticular areas [33]. Reciprocal sympathetic connections have been identified by simultaneous injections of PRV and HSV1, demonstrating the existence of sensory feedback circuits [34]. Subsequent injection of the sensory nerve toxin capsaicin in iBAT resulted in impaired cold tolerance [33], indicating that sensory feedback from BAT is required for appropriate thermogenic responses. In conclusion, activation of hypothalamic neurons, all known to be importantly involved in the regulation of food intake and energy expenditure, innervate BAT, suggesting a major role for peripheral metabolic factors in regulating BAT via the hypothalamus.

Hypothalamic Integration of Peripheral Signals and Regulation of Brown Fat Activity

The hypothalamus senses and integrates signals from the periphery (e.g., leptin levels) and environment (e.g., cold exposure) and responds by regulating sympathetic outflow towards BAT (Figure 2, Key Figure). Other signaling pathways from the brain towards brown fat include the hypothalamic–pituitary–thyroid (HPT) axis (Box 1) and hypothalamic–pituitary–adrenal (HPA) axis (Box 2). The transcription of genes involved in mitochondrial biosynthesis and thermogenesis is regulated by the thyroid hormones T4 and T3 (i.e., HPT axis) and by adrenocorticotropic hormone (ACTH) and glucocorticoids (i.e., HPA axis).

Peripheral and Central Temperature Sensing

Cold- and warm-sensitive receptors send temperature information from the periphery to the hypothalamic POA, parabrachial nucleus, and the peritrigeminal nucleus [35]. In addition, the POA itself also contains temperature-sensitive neurons, whose responsiveness is in return dependent on skin temperature. Glutamatergic stimulation of the POA results in enhanced thermogenesis by BAT [36], indicating functional connectivity.

Due to their strategic localization, subcutaneous thermoreception by the transient receptor potential (TRP) cation channel family is the result of both ambient temperature and blood flow

Key Figure

Hypothalamic Integration of Peripheral Signals and Regulation of Brown Fat Activity

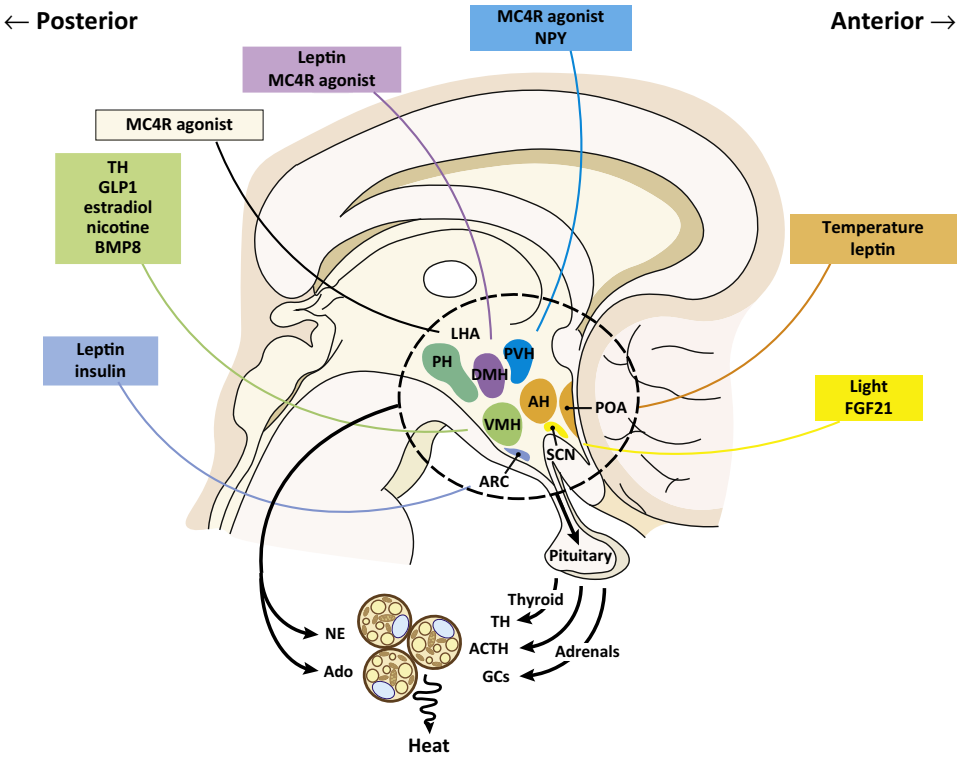

Figure 2. The hypothalamus integrates signals on body temperature, timing, and energy status and responds by regulating the activity of brown fat. The thermogenic activity of brown adipocytes is primarily driven by the sympathetic nervous system upon release of noradrenaline (NE) and adenosine (Ado) in the tissue. Additionally, hypothalamic activation results in pituitary activation and release of adrenocorticotropic hormone (ACTH) and subsequent glucocorticoids (GCs) and thyroid hormone (TH), which directly regulate brown adipose tissue (BAT) activity. Abbreviations: AH, anterior hypothalamus; ARC, arcuate nucleus; BMP8, bone morphogenic protein 8; DMH, dorsomedial hypothalamus; FGF2 fibroblast growth factor 21; GLP1, glucagon-like peptide 1; LHA, lateral hypothalamic area; MC4R, melanocortin 4 receptor; NE, norepinephrine; NPY, neuropeptide Y; PH, posterior hypothalamus; POA, preoptic area; PVH, paraventricular hypothalamus; SCN suprachiasmatic nucleus; VMH, ventromedial hypothalamus.

within the skin. Among the members of the TRP family, TRPM8 is activated by mild cold exposure (<27 °C), and TRPM8-deficient mice exhibit reduced cold tolerance [37]. Conversely, administration of the TRPM8 agonist menthol directly on the skin induces heat production [37]. Interestingly, TRPM8 is also expressed by brown adipocytes and stimulation of brown adipocytes with menthol increases UCP1 expression [38], suggesting a direct temperature sensing and response within brown fat.

It is likely that other TRP channels are also involved in the regulation of brown fat activity. TRPV4 is a warm-sensitive receptor that is highly expressed in WAT, where it acts as negative regulator of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α), UCP1

Box 1. HPT Axis in Brown Fat Function

Upon cold exposure, the hypothalamus releases thyrotropin-releasing hormone (TRH), which stimulates the pituitary to produce thyroid-stimulating hormone (TSH). In turn, thyroid hormones (THs) are released from the thyroid gland in the form of thyroxine (T4), which is transformed into the bioactive hormone triiodothyronine (T3) by the catalytic action of the iodothyronine deiodinase enzymes (D1 and D2) in peripheral tissues, including BAT. In the brown adipocyte, both TSH and THs drive thermogenesis [93]. THs do so by increasing both *Ucp1* gene transcription and stabilization of *Ucp1* mRNA, effects that are synergistic with adrenergic signaling [94].

THs are crucial in sustaining body temperature and the basal metabolic rate can be reduced by as much as 30% in the absence of TH [95]. In addition to direct effects on BAT, THs also potently activate BAT by enhancing sympathetic outflow, since bilateral denervation of the sympathetic nerves innervating BAT or the administration of β-adrenergic receptor antagonists markedly attenuate the thermogenic response of centrally administered TRH [96]. This action may be centered not only in the DMH and/or POA [97], but also within the VMH, where injection of T3 stimulates expression of thermogenic markers in BAT [62]. In conclusion, the HPT axis is critically involved in brown fat thermogenesis; however, the exact contributions of its direct action on BAT and its indirect action via modulating sympathetic outflow need to be unraveled.

expression, and oxygen consumption [39]. Genetic ablation or antagonism of TRPV4 induces elevated thermogenesis and protects from diet-induced obesity, although the possible involvement of the SNS remains unknown.

Similar to TRPV4, TRPV1 is a warm-sensitive receptor that promotes thermoregulatory cooling, at least in part by stimulating hypothalamic vasopressin secretion [40]. Interestingly, TRPV1 is not only expressed in the skin, but also in the intestines. Intestinal TRPV1 agonism by, for example, capsaicin (the pungent ingredient of red pepper) or the unsaturated long-chain fatty acid monoacylglycerol, promotes brown fat thermogenesis and suppresses diet-induced visceral fat accumulation [41]. Thus, direct activation of thermoreceptors by capsaicin and menthol, for example, can be effective strategies to activate BAT.

Circadian Rhythmicity and the Regulation of Brown Fat Activity

Ablation of the SCN [42] or continuous light exposure [43] result in reduced energy expenditure and enhanced weight gain. The identification of neuroanatomical connections between the SCN and BAT [28] indicates the possible involvement of BAT in associations between disturbed circadian rhythm and obesity. Supporting this hypothesis, neurons in the SCN become activated upon cold exposure [44] and injection of glutamate directly into the SCN increases BAT thermogenesis in rats [45]. In addition, it was recently shown that prolonged daily light exposure not only attenuated circadian rhythmicity, but also reduced sympathetic outflow towards BAT, accompanied by decreased BAT activity and increased body fat mass in mice [46]. Cohort studies suggest that, in humans, BAT activity is also physiologically regulated by the biological clock. The detectability of BAT by [^{18}F]FDG-PET-CT imaging at room temperature follows a circannual cycle [47,48], with low detectability of BAT during summer (i.e., long day) compared with winter (i.e., short day). Although differences in outside temperature over the year would be a likely explanation for this phenomenon, the detectability of BAT showed a stronger correlation with day length than with outside temperature [47]. It is tempting to speculate that this seasonal adaptation of BAT activity to day length, which is relevant irrespective of whether the animal is nocturnal or diurnal, precedes changes in temperature and thereby prepares the body for upcoming changes in ambient temperature.

Fibroblast growth factor 21 (FGF21) is a hormone released by the liver and WAT upon various nutrient stresses, such as starvation and by BAT upon cold exposure. Central administration of FGF21 enhanced energy expenditure and prevented weight gain at least partly via activation of BAT and browning of WAT [49–51]. In addition, FGF21 alters circadian behavior, and genetic ablation of the co-receptor β-klotho in the SCN and dorsal vagal complex (DVC) reversed the

Box 2. HPA Axis in Brown Fat Function

Glucocorticoids (GCs) are steroid hormones released by the adrenals upon stimulation by adrenocorticotropic hormone (ACTH) during a stress response. Several studies show strong inhibitory effects of GCs on BAT activity. These may act directly on BAT, by transcriptional repression of *Ucp1* and interference with adrenergic signaling, or indirectly via inhibition of sympathetic outflow from the hypothalamus. Interestingly, in contrast to GCs, ACTH increases BAT activation, likely via stimulation of cyclic (c)AMP production [98].

In general, stress induces activation of BAT, predominantly through activation of the SNS. However, it may also involve corticotropin-releasing factor (CRF)-induced ACTH release. Another possibility for a generalized stress effect on BAT is the direct effect of (hypothalamic) CRF on sympathetic outflow towards BAT [99]. In turn, GCs can subsequently inhibit BAT activity directly or by its negative feedback on the CRF and ACTH release. The underlying mechanism of GC-induced inhibition of BAT activity and the differential effect of ACTH and GCs on BAT remain unclear and need to be further investigated. Conceptually, it is of note that the opposite effects of early (norepinephrine, ACTH) and late (GCs) stress responsive factors are seen in other systems, such as in the infection–inflammation–GC-dependent anti-inflammation process [100].

Given that obesity dampens the cortisol rhythm [101] and is associated with lowered BAT activity, high-fat diet-induced flattening of the cortisol rhythm may be causally involved in the reduction of BAT activity. Whereas under basal conditions the high-affinity mineralocorticoid receptor (MR) is not occupied by cortisol, flattening of the cortisol rhythm leads to continuous activation of the MR. In addition to the GC receptor (GR), MR is also expressed in BAT and, although *in vivo* studies are lacking, *in vitro* studies show inhibiting effects of MR on BAT activity [102]. Therefore, it has been speculated that MR antagonists represent a combined therapy for both hypertension and obesity.

metabolic effects of FGF21 [49], suggesting that FGF21 acts in the SCN through the modulation of circadian rhythmicity.

Regulation of Brown Fat Activity by the Melanocortin System

The central melanocortin system is crucial in the regulation of food intake and energy expenditure. Inhibition of melanocortin 3/4 receptor (MC3/4R) signaling reduced sympathetic outflow towards BAT and BAT activity [2], while activation of the melanocortin system by intracerebroventricular (ICV) administration of MC3/4R agonists (e.g., melanotan II [52], insulin [53], and leptin [54]) enhanced sympathetic outflow and activation of BAT. Restoration of MC4R expression specifically in the LHA in MC4R-deficient mice restored BAT activity and glucose intolerance [55]. However, the PVH [56] and DMH [54] have also been implemented in MC4R-mediated BAT activation. For example, injection of PRV in iBAT of MC4R-GFP-expressing mice suggested MC4R-expressing neuronal populations, among others in the PVN, to be connected to iBAT [57]. Neuropeptide Y (NPY), expressed by neurons in the arcuate nucleus upon fasting and known to inhibit MC4R neurons, regulates BAT function through relay in the PVH [58]. Together, these studies suggest that reduced BAT activity underlies at least part of the obese phenotype in MC4R-deficient individuals [59].

Integration of Peripheral Signals within the VMH through AMPK Activity

The brain directly monitors energy status of the body. High energy availability drives *de novo* lipogenesis not only in metabolic organs, but also in the hypothalamus, yielding increased levels of malonyl coenzyme A (CoA) and long-chain fatty acyl-CoAs that signal the need to reduce food intake. Correspondingly, ICV administration of long-chain fatty acids, such as oleic acid, reduces food intake [60]. Conversely, ICV administration of inhibitors of fatty acid synthase (FAS) has a profound orexigenic effect and may increase energy expenditure because only part of the body weight loss could be explained by a reduction in food intake [61]. Interestingly, fatty acid synthesis is tightly regulated by 5′-AMP-activated protein kinase (AMPK), which is a cellular energy sensor that is active upon low energy availability (i.e., high AMP/ATP). Stereotaxic delivery of a dominant-negative AMPKα into the VMH increased malonyl-CoA levels in the ventral hypothalamus and induced weight lost [62]. This mechanism also mediates at least part of the glucagon-like peptide 1 (GLP1)-mediated reduction in food intake and increase in energy expenditure through BAT activation [63,64]. GLP1 signaling lowers hypothalamic AMPK activity,

thereby increasing neuronal fatty acid synthesis, resulting in decreased expression of orexigenic peptides and increased expression of anorexigenic peptides. Consistent with this pathway, both ICV administration of AICAR (a potent AMPK activator) and an adenoviral vector overexpressing constitutively active AMPK diminished weight loss induced by the GLP-1 analog liraglutide [63].

Other potent molecules that activate BAT through enhancing sympathetic outflow include thyroid hormone [62], estradiol [65], nicotine [66], and bone morphogenetic protein 8 [67]. Evidence for the exact neural pathways involved is limited, but most likely signaling is processed through the VMH. Silencing of the estrogen receptor α specifically in the VMH reduced BAT activity [68] and the reduction in body weight upon T3 administration could largely be prevented by ablation of the thyroid hormone receptor in the VMH [62]. Inhibition of *de novo* lipogenesis in the hypothalamus via ICV administration of an ACC inhibitor reversed weight loss in hyperthyroid rats, pointing towards a mechanism involving regulation by AMPK [62]. Indeed, stereotaxic delivery of the constitutively active AMPKα resulted in weight gain in hyperthyroid rats, but not in euthyroid rats. Similarly, genetic activation of AMPK in the VMH prevented estradiol- as well as nicotine-induced increase in BAT thermogenesis and weight loss [65,66].

Thus, the VMH integrates peripheral signals through AMPK activity and subsequently regulates BAT activity. By contrast, injection of PRV in iBAT only resulted in a few infections in the VMH of hamsters [28] and not in rats [29,30]. One could speculate that AMPK propagates its signals through expression of (an)orexigenic neuropeptides, rather than neurotransmitters.

Interventional and Pharmacological Strategies to Enhance Human Brown Fat Activation

In 2009, three research groups in parallel demonstrated the presence of functional BAT in human adults [3–5]. Mild acute cold exposure revealed uptake of [^{18}F]FDG in the supraclavicular region [4,5]. Daily cold (10 °C) exposure of 2 h for 4 weeks not only increased BAT volume and activity, but also doubled cold-induced energy expenditure [69]. Furthermore, daily mild cold (17 °C) exposure of 2 h for 6 weeks resulted in a decrease in body fat mass [70]. Thus, chronic cold exposure recruits and activates human BAT, but it may be difficult to achieve increased exposure to cold in daily life. A straightforward solution would be to use sympathomimetics. Conflicting reports have been published with respect to the use of these and BAT activation in humans. Oral administration of the non-selective β-adrenergic receptor agonist ephedrine (2.5 mg/kg) activated BAT, as assessed by [^{18}F]FDG PET/CT, in lean but not obese individuals [71]. By contrast, a single intramuscular injection of ephedrine (1 mg/kg) [72] or systemic infusion of isoprenaline (up to 24 ng/kg lean mass/min for 55 min) [73] did not increase [^{18}F]FDG uptake by BAT. Recently, it was demonstrated that a single oral dose (200 mg) of the β3-adrenergic receptor agonist mirabegron was able to mimic the effects of cold-induced BAT activation on [^{18}F]FDG uptake by BAT and energy expenditure [23]. Despite some off-target effects on heart rate and blood pressure, this was the first study demonstrating the potency of a β3- adrenergic receptor agonist to activate human BAT thermogenesis.

Serum FGF21 levels are associated with BAT activity in humans [74], and rodent models have demonstrated metabolic benefits upon FGF21 administration [49–51], although mostly independent of BAT activation [75]. By contrast, serum levels of FGF21 are elevated in obesity and are even higher in metabolic unhealthy obesity [76]. A clinical trial studying the effects of LY2405319, a FGF21 analog, in obese human subjects with T2DM reported improvements in dyslipidemia and a reduction in body weight [77]. Unfortunately, LY2405319 did not reach its primary outcome, namely a reduction in basal glucose levels and, therefore, was not developed further, and direct effects on BAT activation were not investigated. Nevertheless, these data are promising and FGF21 still holds potential as therapeutic target in the treatment of obesity.

Among other compounds implemented in the treatment of T2DM is the GLP1 analog liraglutide that, in preclinical studies, has been shown to activate BAT and to induce browning of WAT via increased SNS outflow [63]. Liraglutide failed to increase total energy expenditure in 4- and 8-week trials [78,79], but increased energy expenditure resulting in weight loss in a 1- and 2-year trial [63,80]. In addition, dipeptidyl peptidase 4 (DPP4)-inhibitors that prevent the breakdown of GLP1 also activate brown fat of mice [81] and appear to improve glucose metabolism [82] and lower body fat in humans [83]. Co-treatment of obese mice with liraglutide and RM-493, which is the only MC4R agonist currently in clinical trials, amplified the metabolic benefits [84]. Subcutaneous infusion of RM-493 in obese subjects for 72 h increased resting energy expenditure by 6.4% [85], underscoring the potency of targeting the melanocortin system.

TRP channels are expressed throughout the body, including the intestines. Dietary menthol-induced activation of intestinal TRPM8 increased the core temperatures in mice [38]. Capsinoids, which are capsaicin-like compounds found in a nonpungent type of red pepper, were as potent as capsaicin in increasing sympathetic nerve activity, thermogenesis, energy expenditure, and fat oxidation, and in reducing body fat in both small rodents and humans [86,87].

Brown fat-targeted therapeutic approaches hold great promise in the treatment of human obesity. Further characterization of human brown fat and the contribution of the SNS to it would be helpful to develop optimal activation strategies.

Concluding Remarks

The CNS is an important regulator of energy balance. The fact that many of the systems involved exert multiple modes of action, including the regulation of energy intake and brown fat activation, makes them attractive and powerful targets for obesity and related disorders, such as hyperglycemia, a precursor of T2DM. Although pharmaceutical targeting of the brain appears to be difficult in humans, one may circumvent this by improving circadian rhythmicity, acclimatization to cold, and the use of sympathomimetic compounds or small molecules that enhance peripheral noradrenergic signaling in BAT. Additionally, several unique approaches have been developed, including combinatorial compounds (targeting multiple receptors simultaneously or targeted delivery) [88,89] and nanoparticles (facilitating delivery to the brain) [90] to specifically target hypothalamic sites, and application of electrical field stimulation to the surface of BAT to mimic SNS outflow [91].

Preclinical studies have shown that BAT activation protects against obesity, dyslipidemia, and even atherosclerosis. So far, only a few studies have explored the metabolic benefits of BAT activation in humans, resulting in many questions remaining (see Outstanding Questions). The translation of preclinical studies to humans is limited by the lack of non-invasive measurements of brown fat activity. The identification of biomarkers and development of other non-invasive measures for brown fat activity (e.g., metabolic magnetic resonance imaging) would be a widely applicable alternative approach.

It has been estimated that brown fat volume in human adults is about 100–200 g and that nonshivering thermogenesis accounts for only 15% of resting energy expenditure [92]. However, small but consistent changes in energy expenditure will ultimately determine body weight and disease, and, therefore, activation of brown fat and/or browning of white fat hold great promise in the treatment of obesity and related disorders.

Outstanding Questions

What is the relevance of brown fat for the human adult body?

Is pharmaceutical targeting of human brown fat a valuable strategy to reverse obesity and related disorders?

How important is the SNS for human brown fat activity?

Does reduced brown fat activity contribute to the obese phenotype in MC4R-deficient individuals?

Is brown fat involved in the association between disturbed circadian rhythmicity (e.g., due to shift work or light pollution) and human obesity?

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